

Effect of Caffeic Acid and Low-Power Laser Light Co-Exposure on Viability of *Pseudomonas aeruginosa*

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Background: The resistance of *Pseudomonas aeruginosa* to antibiotics is a big problem, especially in burns and wound infections. Laser irradiation affects microorganisms by denaturing their proteins, which involves changes in the chemical or physical properties of the protein.

Objectives: The aim of this study was to investigate the effect of caffeic acid and low-power laser light co-exposure on *Pseudomonas aeruginosa* isolated from burn wounds.

Materials and Methods: Ten bacterial samples were collected from patients with burn wound infections at Shahid Motahari medical center of Tehran. The He-Ne laser was used in this study with output power of 2 mW.

Results: The data significantly indicated that both the caffeic acid and laser treatment alone reduced the number of colony-forming units compared to control cultures. Co-exposure of bacterial suspensions to caffeic acid and laser at three time points showed the following number of colony-forming units 240.23 ± 60.15 , 148.13 ± 52.66 and 84.57 ± 35 , respectively. The best concentrations of caffeic acid to achieve countable colonies were 1.5 and 1.75 mM. At the concentration of 1.5 mM of caffeic acid, the number of colonies significantly reduced to 280.78 ± 59 ($P = 0.008$) while at 1.75 mM the number of colonies reduced to 234.07 ± 72.28 ($P = 0.0001$).

Conclusions: Caffeic acid treatment reduced bacterial growth and resulted in a decreased number of colony formation. The simultaneous effect of caffeic acid and laser at three time courses showed a synergic effect in reducing colony formation compared to the control and caffeic acid, and laser alone.

Keywords: Caffeic Acid; Laser Therapy; Low-Level; Minimum Inhibitor Concentration; *Pseudomonas aeruginosa*

1. Background

Pseudomonas aeruginosa is a non-fermentative, aerobic, gram-negative rod that normally lives in moist environments (1, 2). *Pseudomonas aeruginosa* is typically an opportunistic pathogen that seldom causes disease in healthy subjects. Normally, for an infection to occur, some disruption of physical barriers (skin or mucous membranes) or an underlying dysfunction of immune defense mechanisms, such as neutropenia, is necessary (3). The virulence mechanisms of *P. aeruginosa* are complex and only partially understood. Adherence mediated by pili and other adhesions appear to be important for the colonization of mucous membranes and other surfaces (4, 5). Furthermore, the production of a mucoid exopolysaccharide matrix that surrounds the cells and anchors them to each other and to the environment is important for growth as a biofilm, in which the bacterial cells are protected from the host innate and immune defenses and are overall less susceptible to antibiotics (6-9). A role for tissue damage and invasion has been recognized for a number of products secreted by *P. aeruginosa*, including elastase, alkaline

protease, cytotoxin, phospholipase C and rhamnolipid (10, 11). Caffeic acid phenethyl esters (CAPE) (2-phenyl ethyl 3 (3, 4-dihydroxyphenyl) -2-propenoate), exhibits a broad spectrum of biological activities including antibacterial, anti-inflammatory, antiviral, antiatherosclerotic, antiproliferative, neuroprotective, and antitumoral actions (12, 13). Phenethyl caffeic acid esters, similar to ferulic and chlorogenic acids, show antibacterial, anti-mutagenic, and antiviral activities (14). Several studies have shown the antibacterial effects of these components, e.g. diphenyl esters of caffeic acid act as highly effective antimicrobial agents against staphylococcal bacteria, *Bacillus subtilis*, and *Pseudomonas aeruginosa* (15). Low power laser irradiation (LPLI) has been used for a variety of clinical applications where it is thought to promote certain processes without inducing any thermal effects. Studies have demonstrated that laser-powered treatment will be useful for the treatment of many cases of infection caused by bacteria such as *P. aeruginosa* (16). Thus, we aimed to investigate the effect of low-power laser irradiation (LPLI)